because cells with aberrations are less likely to continue mitosis, but the decrease in frequency of aberration in control roots fixed when about 1.5 cm long is not statistically significant. The longer roots grown in the cycasin solution had a significantly higher frequency of aberration than the shorter roots; this may result from the longer duration of treatment or from differential sensitivity during the nuclear cycle. In the longer root tips the cycasin produced about as many aberrations as irradiation of 0.2-cm roots with 200 r of gamma rays.

It has been postulated that the carcinogenic quality of cycasin may relate to action of the aglycone as an alkylating agent in forming diazomethane in vivo (*8*). Evidence from germfree animals and injection experiments indicates that it is probably the unstable aglycone rather than cycasin itself that is the active toxic and carcinogenic agent (*1, 3*). Many plants are known to contain emulsins of the type that split cycasin. It may be that cycasin affects *Allium* chromosomes by way of its slow hydrolysis to form methylazoxymethanol.

The finding that cycasin induces breaks in *Allium* chromosomes raises questions concerning both the nature of the resistance of cycad plants to cycasin and the radiomimetic effects of other carcinogenic agents.

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References and Notes

1. G. L. Laqueur, *Federation Proc.* **23**, 1386 (1964).
2. O. Mickelson, M. G. Whiting, L. T. Kurland, *J. Nat. Cancer Inst.* **31**, 919 (1963).
3. A. Kobayashi and H. Matsumoto, *Federation Proc.* **23**, 1354 (1964).
4. T. Nagahama, *Bull. Fac. Agr. Kagoshima Univ. 14*, p. 1 (1964).
5. C. Auerbach, *Cold Spring Harbor Symp. Quant. Biol.* **16**, 199 (1951).
6. Cycasin was obtained by courtesy of M. G. Whiting, NIH.
7. Onion seed donated by Asgrow Seed Co., Twin Falls, Idaho.
8. J. A. Miller, *Federation Proc.* **23**, 1361 (1964).
9. Work supported in part by PHS grant RH 00294.

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Circadian Rhythm in Pineal Serotonin: Effect of Monoamine Oxidase Inhibition and Reserpine

Abstract. *The pineal gland of the rat shows a circadian rhythm in its serotonin content, the amount of serotonin decreasing at night. This decrease can be prevented by inhibiting the action of monoamine oxidase. Reserpine abolishes the circadian rhythm in pineal serotonin in the same manner as does interruption of the sympathetic nervous connections of the central nervous system and the pineal gland. These observations suggest that circadian changes in release and binding of serotonin may occur in the pineal gland, and that a central mechanism in which monoamines participate may control the circadian pineal-serotonin rhythm.*

There are at least two circadian rhythms in the pineal gland of the rat; one is concerned with the enzymatic synthesis of melatonin (*1*), a gonadal inhibitory hormone (*2*), and another, with the pineal content of serotonin, a precursor of melatonin (*3*). The activity of hydroxy-indole-*O*-methyl transferase, the enzyme which synthesizes melatonin, is highest at midnight and lowest at 6 p.m. (*1*). This rhythm is completely exogenous and can be extinguished by blinding the animals or by changing their environmental lighting. The concentration of serotonin in the rat pineal gland varies from a maximum of 60 to 80 ng per milligram (wet weight) at 1 p.m. to a minimum of 10 to 30 ng per milligram at 11 p.m. This rhythm is endogenous and persists for at least 2 weeks in blinded animals and in rats kept in continuous darkness (*4*). The nocturnal fall in the serotonin content of the pineal gland can be prevented by allowing the lights to remain on for an additional 4 hours on a given day (*4*). Both the melatonin (*1*) and serotonin rhythms (*4, 5*) are abolished by severing the sympathetic nerves to the pineal gland.

Several mechanisms could cause the marked rise and fall in the serotonin content of the pineal gland. More serotonin might be formed during the day than at night, or more might be destroyed at night. It is also possible that changes occur in the binding and release of serotonin during the day and night.

We estimated the activity in the pineal gland of 5-hydroxytryptophan decarboxylase, the enzyme which forms serotonin, and of monoamine oxidase, an enzyme which metabolizes serotonin, every 4 hours during a 24-hour period, using groups of 10 rats for each time-point. In this and subsequent experiments Sprague-Dawley female rats (180 to 200 g) were kept in a room at 25°C and were subjected to cycles of 14 hours of light (fluorescent lighting was automatically turned on at 5 a.m.) and 10 hours of darkness (lights turned off at 7 p.m.) for at least 1 week. The activities of 5-hydroxytryptophan decarboxylase (*6*) and monoamine oxidase (*7*) were measured by specific and sensitive methods. There were no changes in either monoamine oxidase or 5-hydroxytryptophan decarboxylase activities during the day or night.

In other experiments we examined the possibility that changes in the synthesis of serotonin in vivo, or in the transport of amino acid precursors into intracellular synthetic sites in the pineal gland, might account for the circadian changes in the serotonin content of the pineal gland. Groups of ten rats each received intraperitoneal injections of 5-hydroxytryptophan (100 mg/kg) or tryptophan (200 mg/kg) at noon or at 10 p.m. and the serotonin content of single pineal glands was measured 1 hour later by a fluorometric method specific for serotonin (*8*). The increment in pineal serotonin content in rats injected with tryptophan (100 ng/mg) and in rats injected with 5-hydroxytryptophan (60 ng/mg) was the same at night and during the day. These experiments suggest that the changes in the serotonin content of the pineal gland are not the consequence of changes in synthesis of this biogenic amine.

Since there are large amounts of serotonin (*3*) and monoamine oxidase (*9*) in the pineal gland and since both serotonin (*10*) and monoamine oxidase (*11*) are highly localized in sympathetic nerve endings in the pineal gland, it seems probable that serotonin is bound and stored in the gland in such a form as to be inaccessible to destruction by monoamine oxidase. Quay (*3*) observed that the nocturnal fall in pineal serotonin is precipitous. He found that between 7 p.m., when the lights were turned off, and 11 p.m. the serotonin content of the pineal gland decreased at a rate of 25 ng per gland per hour to about 20 percent of its value of 7 p.m. The rapid nocturnal decline of

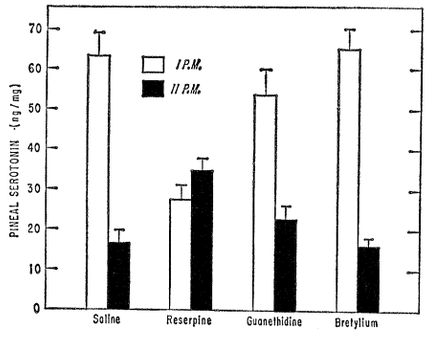

Fig. 1. Effect of reserpine, guanethidine, and bretylium on the circadian rhythm in the serotonin content of the rat pineal gland. Each group contained ten rats. Vertical bars show the magnitude of the standard error of the mean.

pineal serotonin suggests that at night serotonin might be released from a bound form so that it becomes accessible to destruction by monoamine oxidase. If this were the case, inhibition of monoamine oxidase should prevent the destruction of serotonin released at night and eliminate the usual nocturnal decline in serotonin. Moreover, if serotonin were maintained in a bound form during the day, inhibition of monoamine oxidase should not have a marked effect on daytime concentrations of serotonin.

Rats maintained for 7 days on light–dark cycles of 14 : 10 were injected with the monoamine oxidase inhibitor, β-phenylisopropylhydrazine; some were killed at 1 p.m. and others at 11 p.m. Controls were injected with saline (Table 1). In the controls, the concentration of serotonin was more than twice as high at 1 p.m. as at 11 p.m. Treatment with the monoamine oxidase inhibitor did not affect the concentrations of serotonin at 1 p.m. but prevented the nocturnal decline of serotonin. These observations suggest that circadian changes in pineal serotonin content may be due to the release of bound serotonin at night.

The circadian rhythm of the serotonin content of the pineal gland is controlled by information transmitted from the central nervous system to the pineal gland by way of preganglionic fibers to the superior cervical ganglion where they synapse with postganglionic fibers which innervate the pineal gland (*4*). These conclusions were drawn from experiments in which the circadian changes in serotonin in the pineal gland were abolished by decentralization of the superior cervical ganglion, an operation which interrupts the adrenergic connections between the central nervous system and the superior cervical ganglion.

Since the serotonin rhythm of the pineal gland appears to be controlled by adrenergic nerves, peripherally and possibly centrally, we examined the effects on the rhythm of various drugs which affect adrenergic activity. The compounds used were reserpine, a drug which depletes the adrenergic neurotransmitter norepinephrine both centrally and peripherally, and guanethidine (*12*) and bretylium (*13*), both of which block the transmission of sympathetic nerve impulses peripherally. Guanethidine, in addition, depletes norepinephrine peripherally but not centrally (*14*). Rats were given daily intraperitoneal injections of bretylium (20 mg/kg), guanethidine (20 mg/kg), or reserpine (1 mg/kg) for 3 days. Control animals were injected with saline. Rats were killed on the 3rd day at 1 p.m. or 11 p.m. and the serotonin content of the pineal glands was measured (Fig. 1). Bretylium and guanethidine treatment had no effect on serotonin concentrations during the day or night. Reserpine, however, abolished the circadian changes in the serotonin content of the pineal gland, the concentration at 1 p.m. being lowered and that at 11 p.m. being raised.

In subsequent experiments, the serotonin content of the pineal gland of reserpine-treated rats was measured at 4-hour intervals over a 24-hour period and found to be the same at all time points. Pletscher *et al.* (*15*) and many other investigators have shown that reserpine reduces the serotonin content of many tissues. Our results represent the first example of the serotonin content of a tissue being increased after the administration of reserpine, although this elevation is detected only at night. This effect is similar to that observed in rats subjected to superior cervical ganglionectomy or decentralization of the superior cervical ganglia (*4*).

Bretylium and guanethidine did not affect the serotonin rhythm in the pineal gland. Maintenance of the rhythm requires intact peripheral sympathetic innervation to the pineal gland (*4*). The failure of bretylium and guanethidine to alter the rhythm suggests that these drugs may not alter transmission of impulses in sympathetic nerves innervating the pineal gland in the same way as they affect other peripheral sympathetic nerves.

Table 1. Effect of a monoamine oxidase inhibitor on the circadian rhythm in the serotonin content of the rat pineal gland. Lights were kept on from 5 a.m. to 7 p.m. Rats were given intraperitoneal injections of the monoamine oxidase inhibitor, β-phenylisopropylhydrazine (5 mg/kg), 24 hours and 12 hours before being killed. Each group contained ten rats. Results are expressed as the mean ± standard error of the mean.

Time rats were killed	Serotonin content of pineal gland (ng/mg)
Rats injected with saline	
1 p.m.	62 ± 8.1
11 p.m.	29 ± 3.3*
Rats injected with β-phenylisopropylhydrazine	
1 p.m.	71 ± 5.1
11 p.m.	63 ± 9.3

* Differs from all other groups $p < .001$.

The abolition of the rhythm in the serotonin content of the pineal gland by decentralization of adrenergic nerves (*4*) has shown that the rhythm is controlled by the central nervous system. The present finding that reserpine, a drug which depletes central as well as peripheral monoamines, similarly extinguishes the serotonin rhythm suggests that the rhythm is regulated by a mechanism involving brain monoamines, possibly by a central adrenergic mechanism. Our data, however, do not exclude the possibility that reserpine might alter the pineal serotonin rhythm solely by its peripheral actions. Moreover, our observations cannot rule out the possibility that reserpine shifts the pineal serotonin rhythm to a period greater than 24 hours, as observed by Halberg (*16*) in thermovariance spectrums of human subjects treated with reserpine.

The serotonin rhythm of the pineal gland has many characteristics in common with other biological circadian rhythms. These rhythms are endogenous (*4, 17*), can be cued by environmental lighting (*4, 18*), and can be reversed by changes in the lighting schedule (*17, 19*). It is, therefore, possible that other biological circadian rhythms, such as those concerned with adrenal secretion and running activity, might also be controlled by a similar central mechanism.

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References and Notes

1. J. Axelrod, R. J. Wurtman, S. H. Snyder, *J. Biol. Chem.* **240**, 949 (1965).
2. R. J. Wurtman, J. Axelrod, E. W. Chu, *Science* **141**, 277 (1963).
3. W. B. Quay, *Gen. Comp. Endocrinol.* **1**, 3 (1963).
4. S. H. Snyder, M. Zweig, J. Axelrod, J. E. Fischer, *Proc. Natl. Acad. Sci. U.S.* **53**, 301 (1965); S. H. Snyder, M. Zweig, J. Axelrod, *Life Sci.* **3**, 1175 (1964).
5. V. M. Fiske, *Science* **146**, 253 (1964).
6. S. H. Snyder and J. Axelrod, *Biochem. Pharmacol.* **13**, 805 (1964).
7. R. J. Wurtman and J. Axelrod, *ibid.* **12**, 1439 (1963).
8. S. H. Snyder, J. Axelrod, M. Zweig, *Biochem. Pharmacol.* **14**, 831 (1965).
9. R. J. Wurtman, J. Axelrod, L. Phillips, *Science* **142**, 1071 (1963).
10. A. Bertler, B. Falck, C. Owman, *Kgl. Fysiograf. Sallskap. Lund. Forh.* **33**(2), 13 (1962).
11. S. H. Snyder, J. E. Fischer, J. Axelrod, *Biochem. Pharmacol.* **14**, 803 (1965).
12. A. L. A. Boura and A. F. Green, *Brit. J. Pharmacol.* **14**, 536 (1959).
13. R. A. Maxwell, R. P. Mull, A. J. Plummer, *Experientia* **15**, 267 (1959).
14. R. Cass and T. L. B. Spriggs, *Brit. J. Pharmacol.* **17**, 442 (1961).
15. A. Pletscher, P. A. Shore, B. B. Brodie, *Science* **122**, 374 (1955).
16. F. Halberg, *Proc. Roy. Soc. Med.* **56**, 253 (1963).
17. M. S. Johnson, *J. Exptl. Zool.* **82**, 315 (1939).
18. F. Halberg, E. Halberg, C. P. Barnum, J. Bittner, in *Photoperiodism and Related Phenomena in Plants and Animals*, AAAS Publ. No. 55, R. B. Withrow, Ed. (AAAS, Washington, D.C., 1959), p. 803.
19. S. H. Snyder and J. Axelrod, unpublished observations.

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Biochemical Polymorphism in Ants

Abstract. *Ants of different sexes and castes produce different odorous compounds. In* Pheidole fallax *Mayr, soldier ants produce an indole base, probably skatole, whereas minor workers produce a trail substance. Males of certain species of* Lasius *and* Acanthomyops *produce mixtures of terpenes and an indole base. These mixtures are discharged during mating flights and probably are used as mating pheromones. The terpene mixtures are qualitatively similar, but each species produces a blend of distinctive proportions. Citronellol and 2,6-dimethyl-5-hepten-1-ol have been identified in the mixtures by gas chromatography and mass spectrometry.*

The various sexes and castes of individual species of social insects often produce different pheromones, and such differences are fundamental to social behavior. As a specific example, only the queen honey bee produces 9-keto-2-decenoic acid, which is a sex pheromone (*1*) and which plays a crucial role in the organization of worker behavior (*2*). Extensive observations of behavior indirectly indicate the widespread occurrence of similar phenomena (*3–5*), which we propose for convenience to term "biochemical polymorphism," since the occurrence of these different molecules must reflect differences in biochemical machinery or in the control of biochemical machinery. We now describe two new examples from the ants.

In Trinidad, West Indies, one of us (E.O.W.) noted that agitated soldiers of the myrmicine ant *Pheidole fallax* Mayr produced a fecal odor. By dissections the odor was quickly localized in the poison gland vesicle, which was also found to be peculiarly hypertrophied in the soldier caste and occupied approximately one-third of the entire abdominal cavity. The odor was characteristic of indole compounds, such as skatole. Paper chromatography of ant extracts (*6*) gave a spot that corresponded in mobility and in color with Erhlich reagent to skatole. The material also gave the Steensma test characteristic of skatole, but not of indole (*7*).

Minor workers of *P. fallax* produce no detectable amounts of the indole compound, and their poison gland vesicle is of normal proportions. On the other hand, minor workers lay odor trails, whereas soldiers do not. The trail pheromone, which is a volatile, true attractant, is produced by Dufour's gland and is disseminated through the sting. In the minor worker the Dufour's gland is exceptionally large in comparison with the same organ in workers of other myrmicine species of comparable size; it is even larger than the associated poison vesicle. In the soldier, the Dufour's gland is either greatly reduced or absent. Moreover, no trail-laying behavior on the part of soldiers was ever observed in field observations in Trinidad, and no detectable trace of the trail substance could be found in the abdomen of the soldier ant by means of the artificial-trail bioassay (*3*), although the soldiers readily follow the trails laid by minor workers. Here, then, is an instance in which one of two castes produces distinct chemical substances not produced by the other caste.

We have also observed that male ants of two members of the genus *Lasius* contain strongly odorous substances, which the workers appear to lack. Furthermore, although workers of *Acanthomyops claviger* (Roger) produce monoterpene aldehydes of strong odor (*8*), which function both as defensive substances and alarm pheromones (*4*), the males produce a mixture of compounds with an odor distinctly different from that of the workers.

Male ants of the species *Lasius neoniger* Emery, *Lasius alienus* (Förster), and *Acanthomyops claviger* (Roger) were collected at nests in the vicinity of Lexington, Massachusetts, while the ants were exhibiting preflight behavior. The crushed ants had two distinct odors. At first one could detect the sweet odor of volatile terpenes and then the fecal odor of an indole compound. Simple dissection showed that both odors originated in the head, whereas the thorax and the abdomen were without odor. The odorous substances were further localized in the reservoir of the mandibular gland, which, prior to the nuptial flights, are relatively large structures, turgid with volatile liquids. Males of *Lasius neoniger*, captured around electric lights in the evening after the mating flights, had little or no odor. Possibly these substances are discharged by the males during the flights, and they might serve as sex pheromones.

The amount of indole compound in

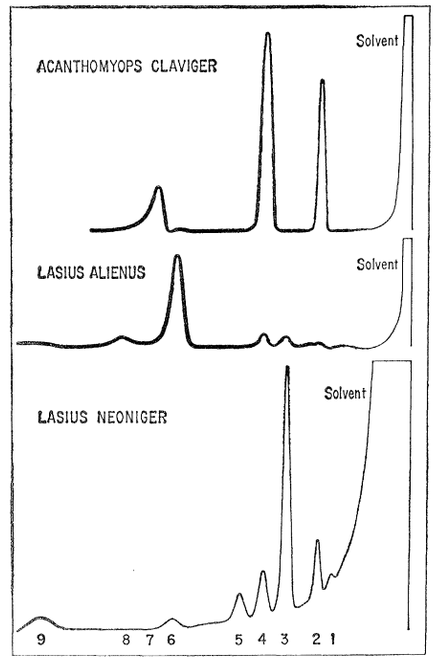

Fig. 1. Gas chromatograms of volatile compounds from male ants. The column, 1.8 m by 0.6 cm, was packed with 10 percent polydiethylene glycol succinate on Chromosorb W. The temperature was 125°C and the argon pressure, 0.7 atm (Research Specialities model 600).